Images of Anthrax

A Team Approach

PHOTOS BY PAUL ROBERTS, MSOE

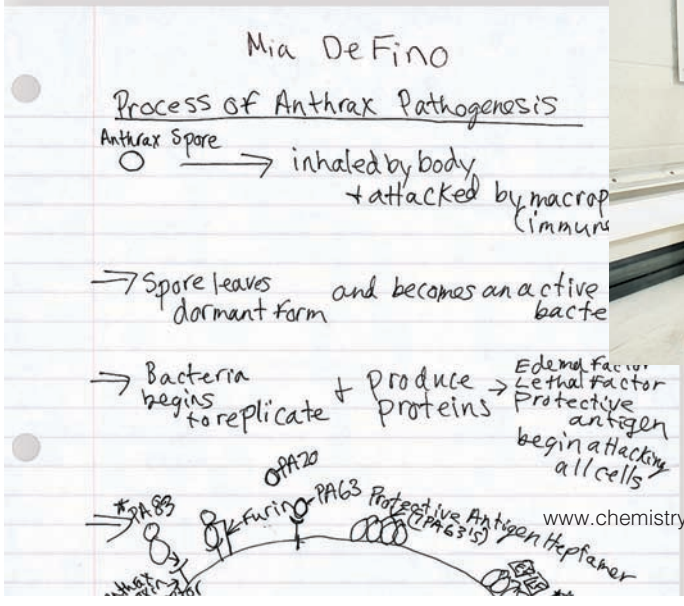
By Jonathan Knopp

The nation watched in horror as the catastrophic events of September 11, 2001, caused loss of life and massive destruction in a mere instant. Then, within only a few weeks, came the first of five reports of seemingly random deaths traced to anthrax—an infection thought to have long disappeared from the population. Gradually, news of anthrax contamination and exposures spread over ever-widening geographic areas.

These unforeseen acts of bioterrorism forever changed the lives of three Milwaukee teenagers as they began their senior year at Riverside University High School. In late summer 2001, Mia Defino, Mike Poliak, and Justin Snowden only knew that, thanks to their science teacher Jeff Anderson, they were looking forward to an interesting science internship at the Milwaukee School of Engineering (MSOE). Working as a team, their challenge was to select a protein from a database and make a molecular model. Why not choose some of the key proteins associated with this mysterious anthrax bacteria that was all over the news? At this point, no one dreamed that by mid-school-year, they would be conversing with some of the world's leading anthrax researchers and producing an important research tool in the nation's war against bioterrorism. Amid schoolwork and extracurricular activities like football and jobs, the students began reading research articles and any information they could obtain about anthrax. Their thoughts and their conversations centered on topics like the interaction of anthrax with cell surface receptors, the mechanism of attack, and details of cell destruction by anthrax. As former students in Anderson's Advanced Placement biology class, they relied on concepts previously learned. Mia recalls that AP biology helped a lot in the project. "It made us familiar with the vocabulary and some important microbiology topics such as the lytic cycle, endocytosis, protein synthesis, and just proteins in general."

Before long, they identified three key anthrax-related proteins that they wanted to model. Before 6:00 a.m. on school days, after school, and sometimes on weekends, the three traveled to MSOE to learn the process of biomolecular modeling. Students and teacher soon became known around the lab as "Team Anthrax".

At MSOE, rapid prototyping technology is coupled with computer-aided design to turn out three-dimensional models of molecules. In the automotive industry, engineers have regularly produced precise models of engine parts they design on their computer screens. Recently, this combined technology has been expanded and applied to the biomolecular world by Dr. Tim Herman, director of the Center for Biomolecular Modeling (www.rpc.msoe.edu/cbm) at the Milwaukee School of Engineering. The Protein Data



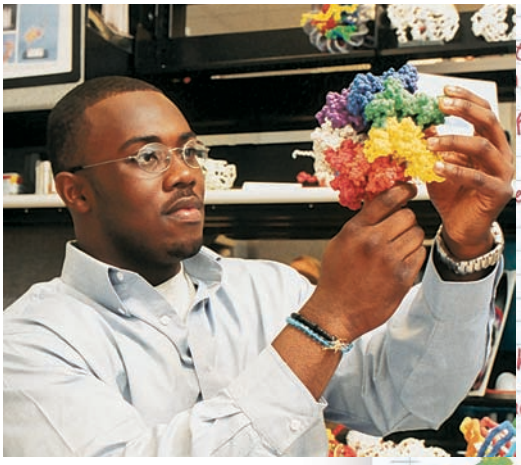
Top photo: Team Anthrax examines protein models. Left to right: Mia Defino, Justin Snowden, and Mike Poliak. Bottom photo: Mia examines a finished model.

Bank (PDB) Web site at **www.rcsb.org/pdb**, contains the spatial x,y,z coordinates that give relative position information for the atoms in any listed protein. These data are contributed by X-ray crystallographers after determining the molecular structures. Today, anyone can freely access the information in the PDB. Atomic coordinate data from the PDB can then be translated through Rasmol, a freely available software program, into a computer image of the molecule.

To build a model, the spatial coordinates guide the way. Additional software at MSOE relays the computer image data to a rapid prototyping machine—the machine that builds the three-dimensional model, one layer at a time (see sidebar below).

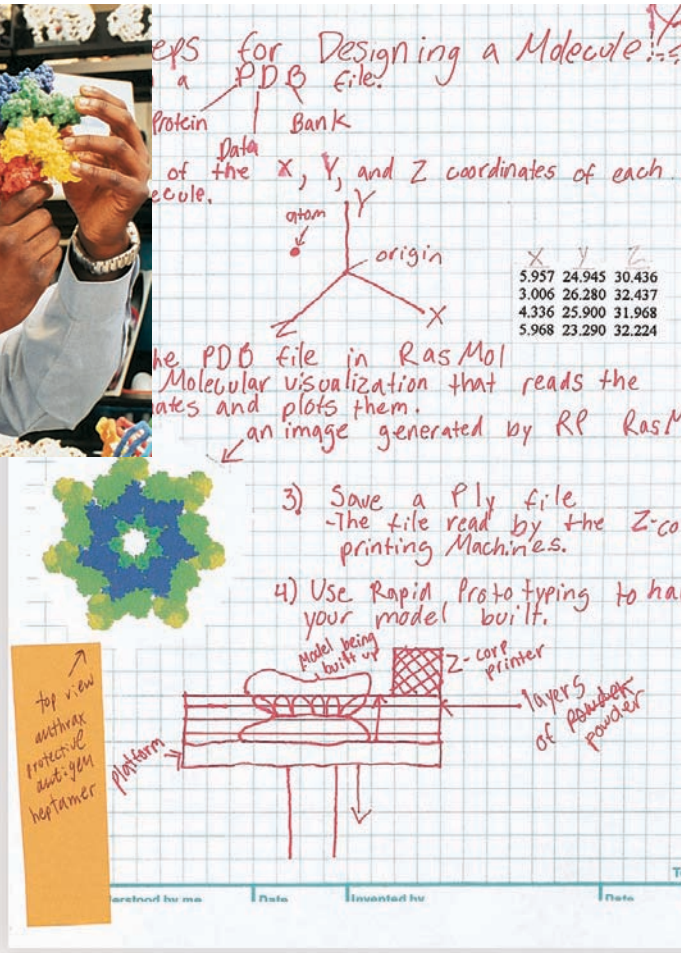
After learning to use these available tools, the students went to work on their selected molecules. They started by carefully examining the molecular structures in the PDB. With Herman's help, the students added monitor lines, additional components to be added as structural supports in the physical model. They removed glitches in the program where the computer perceived hydrogen bonding where none really existed.

Team Anthrax turned out two models representing protective antigen and lethal factor, two of the three known anthrax proteins. About the size of one's fist, the models have been scaled up 17 million times larger than life. And with the production of these models, a whole new phase of engagement began.



Justin: "Someday, I can tell my kids that I was on a high school team that made the world's first models of anthrax protein."

In December 2001, Herman and a colleague at MSOE, Dr. Mike Patrick, were attending a meeting at the Howard Hughes Medical Institute (HHMI) in Washington, DC. At lunch, they were discussing the anthrax models with an HHMI investigator from the University of Chicago who mentioned that his colleague, Dr. Wei Jen Tang, had just solved the structure of another anthrax toxic agent. Herman suggested that Team Anthrax contact Dr. Tang for his assistance. To their delight, Tang agreed to share three years of his research data on the anthrax protein, edema factor—data that were about to be published in *Nature*, one of the world's premier scientific journals.



Justin's notes on designing a molecular model.

NOTEBOOK PAGES COURTESY OF TEAM ANTHRAX

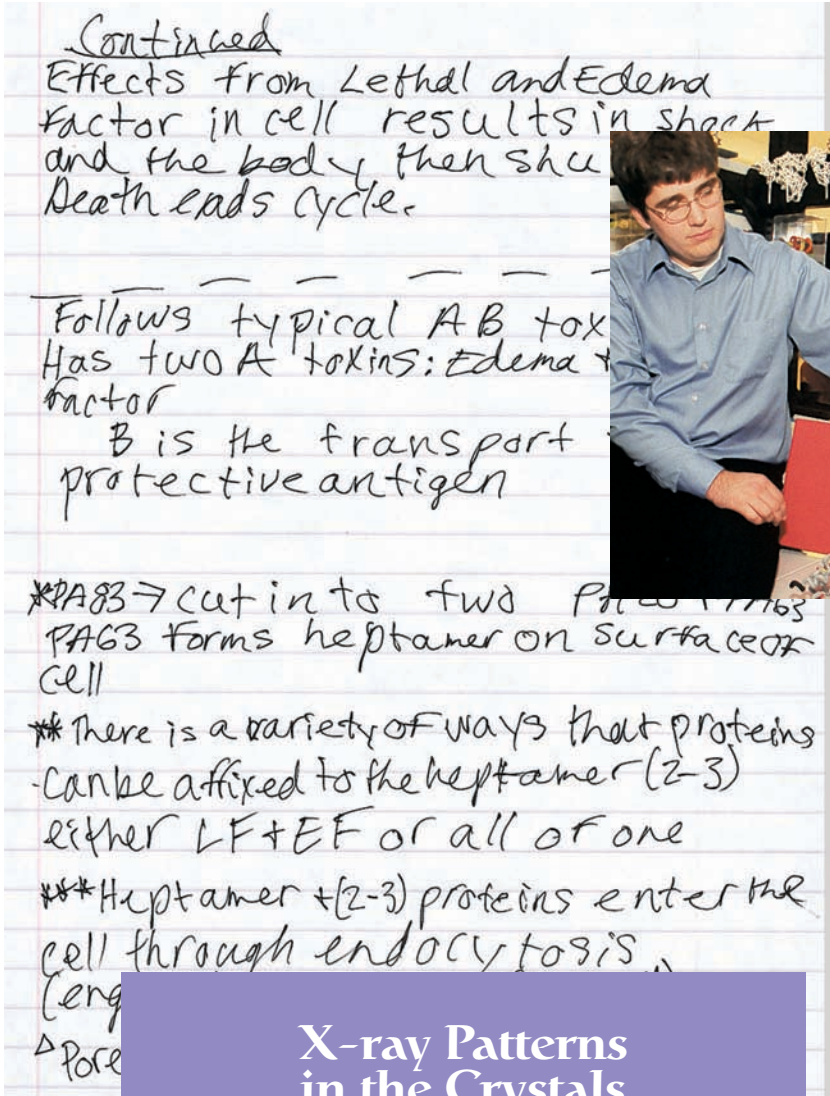
Now, Team Anthrax had all the data needed to make the world's only models of all three known anthrax proteins. Tang was invited to travel to Milwaukee, where the students proudly presented him with a set of models. It was during the visit the team realized they had advanced their own knowledge to the point that they could converse with a leading researcher, sharing insights and asking important questions. The hard work had paid off!

The Team Anthrax story began to get attention in the local and regional press. Mike recently reflected on the impact of the public attention. "The project took a lot of time from chemistry class, and it caused stress because of the public speaking engagements. But now, because of the project, I can talk in front of people."

Making Models by Rapid Prototyping —One Layer at a Time

Rapid prototyping (RP) is an additive manufacturing process by which accurate three-dimensional models are constructed, layer by layer. Since each layer is only three thousandths of an inch thick, RP is actually a slow process, often taking 15–20 hours to complete a model. A Z Corp 3D printer, the rapid prototyping machine used by Team Anthrax, looks like a large automatic washing machine. Layering begins when a scanning arm equipped with an ink jet cartridge sweeps back and forth over a layer of powder leaving a trail of droplets. Each droplet includes glue so that when the droplet contacts the powder, a minuscule solid particle results. After the scanning arm has completed its passage over the layer of powder, the entire powder layer, which is supported on a tray, is lowered by three-thousandths of an inch into a bin. A second arm spreads out another layer of powder to prepare for the next passage of the scanning arm. This layering and gluing sequence repeats over and over for thousands of times. The process resembles the formation of a cave stalagmite on the floor of a slowly descending elevator. After the last passage of the scanning arm, the model is complete. Remove the bin, blow away the free powder with an air gun, and Voila! A model appears! The technician examines the product, completing the model by infiltrating it with resins to harden it.

A completed physical model represents a molecule, enlarged about 17 million times, yet true to the relative positions of the constituent atoms and functional groups.



Mike compares a finished model to its screen image using Rasmol software.

PHOTO BY PAUL ROBERTS, MSOE

The real value of the anthrax models became even clearer to the team when researcher John Young at the University of Wisconsin–Madison invited the students to his laboratory in February 2002. Young, a recognized authority on the anthrax bacterium, coauthored the March 2002 cover story in *Scientific American* entitled "New Antidotes to ANTHRAX" that was just about to appear on newsstands. When given an anthrax protective antigen heptamer model, Dr. Young stopped all conversation and began to inspect the model. Soon, he and his colleagues began to discuss their own work in terms of binding sites readily identifiable on this unique model. Continuing to examine it, Dr. Young held onto the model for the rest of the visit.

Then, Young had an idea. He told the team that he had been invited to speak before a special Congressional hearing on bioterrorism on Capitol Hill in Washington, DC. Could the students make enough copies of the models for him to distribute to members attending the Congressional hearing to facilitate their understanding of anthrax? The team was thrilled. They readily agreed to make 25 protective antigen heptamer models.

And what do their friends think of all this? Their reactions have been "interesting", and, for the most part, positive. According to Mike, "A lot of people came up in the hallway at school saying 'We saw you on TV.' or 'We heard about you on the news.' They weren't sure what it was about except they thought it was cool."

Mia, Mike, and Justin give a lot of credit for the success of the project to their mentors. Anderson, their teacher "was always willing to help us with anything and everything at any time for this project. Dr. Herman and Jennifer Morris at MSOE went above and beyond in offering their help."

Where will Team Anthrax members go from here? For the immediate future, they will be in college. Forty years into the future, Mike hopes to look back and view his Team Anthrax experience as the first step toward his science career. Justin thinks about the emotional impact of the experience, "People will always talk about September 11, and I can tell my kids that I was on a high school team that made the world's first models of anthrax protein."

X-ray Patterns in the Crystals

In the health sciences, radiologists use X-rays for diagnosis and cure. In the world of protein research, crystallographers direct X-rays at protein crystals to understand their structure.

The first task of a crystallographer is to prepare crystals of a given protein to be analyzed. Often, during this difficult task, two versions of the protein crystal are prepared. One version is the natural protein, whereas another is of the same protein infused with atoms such as mercury or selenium to act as markers for comparison. Often less than 0.5 mm on a side, the crystals are suspended in a glass capillary tube and then bombarded by an X-ray beam. Atoms of the crystal, especially the marker atoms, scatter the incoming beam to produce diffraction patterns that are recorded on a photographic plate. The complex patterns are analyzed mathematically, resulting in assigned coordinates for every atom in the protein. Crystallographers are proud to deposit their data on the Protein Data Bank (PDB) maintained by the Brookhaven National Laboratory.

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Jonathan Knopp, a former science teacher in the Milwaukee Public Schools, now works as a consultant in the Center for Biomolecular Modeling at MSOE.
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